Modern Instrumental Methods of Analysis
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Module No. # 02
Lecture No. # 08
Ultraviolet and Visible Spectrophotometry - 4 Instrumentation

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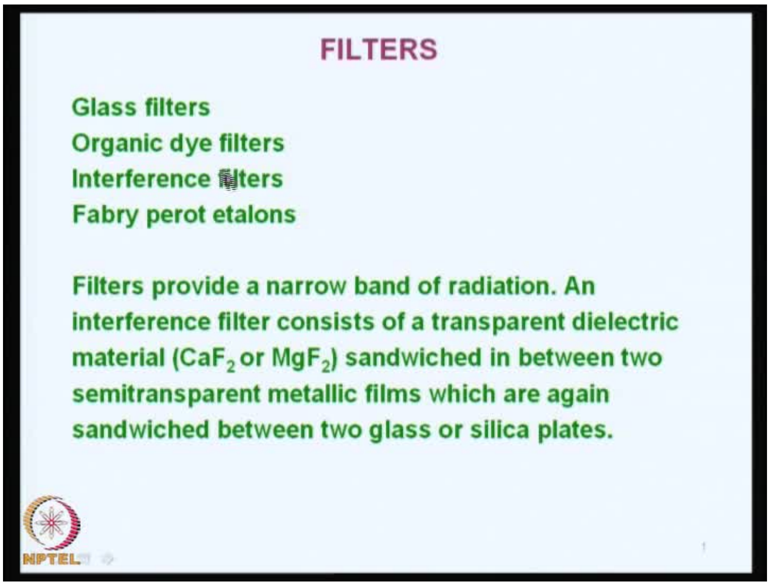

Welcome to the next session for discussion on the instrumentation of spectrophotometers. In the last class, we have studied the sources and we have discussed about tungsten lamp, hydrogen lamps and xenon arc lamps etcetera. And in this class, we will continue our discussions with the other parts of the instruments that are required in a spectrophotometer. For example, what you need next if you take out particular a portion of the radiation from a lamp, it will be giving you complete spectrum. So, you have to select a particular wavelength and selection of the wavelength is done by the filters.

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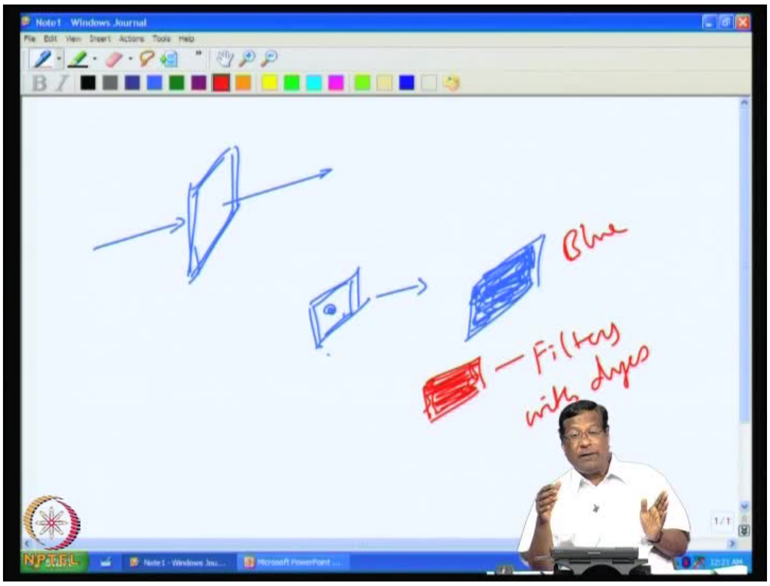

So, basically filters provide a narrow band of radiation. An interference filter, there are difference kinds of filters actually, you can use glass filters, organic dye filters, interference filters and fabry perot etalons. And these glass filters, for example, are simple glass filters like this and they are all colored.

So, you could see that if you use any glass, like a goggle or something like that, you would see only the specific colors that are visible and it would be interesting to use glass filters. But these glass filters do not have the choice of variety of wavelengths. So, they are of limited use and useful only for specific applications.

Now, you can also imagine, take two white glass filters put them together, again you can put two glass filters. And put some amount of dye and then sandwich the dye in between these two. So, the sandwiched dye will look something like this with a colored dye. And this colored dye, you can choose number of colored dyes one with like this or you can do a red one red filter, you can prepare number of dyes like this. These are all glass filters with dyes, and this is also, this is a blue dye. So, like that you can choose different colored dyes for different choice of wavelengths.

Now, again these dyes have got a problem because they will be permitting only particular kind of radiation passing through them. So, it is not very ideal and moreover it will pass about 30 to 35 nanometer of radiations and what we are looking for would be somewhere around 5 or 6 nanometers of band pass width whereas, the band pass width for these things would be around 30 nanometer that is the range for which it will be useful.

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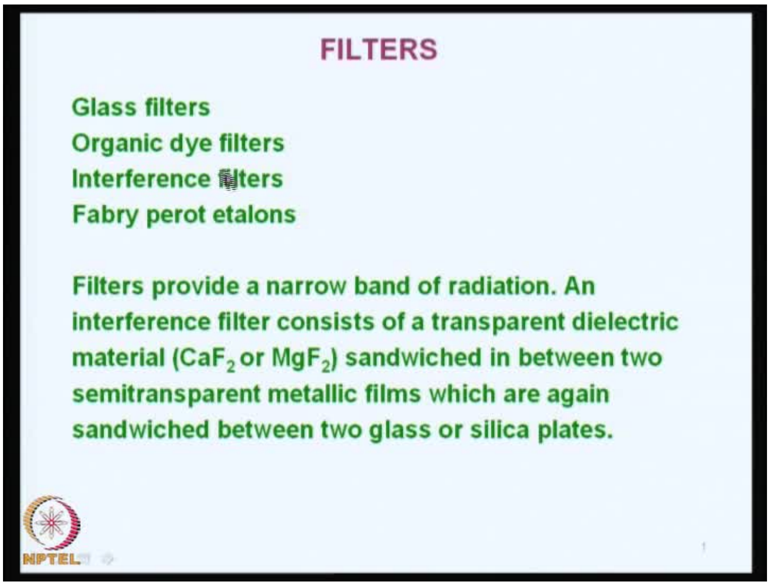

So, we can go for an interference filters. So, what is an interference filter? It consists of a transparent dielectric material for example, calcium fluoride or magnesium fluoride sandwiched between two semi-transparent metallic films, which are again sandwiched between two glass or silica plates.

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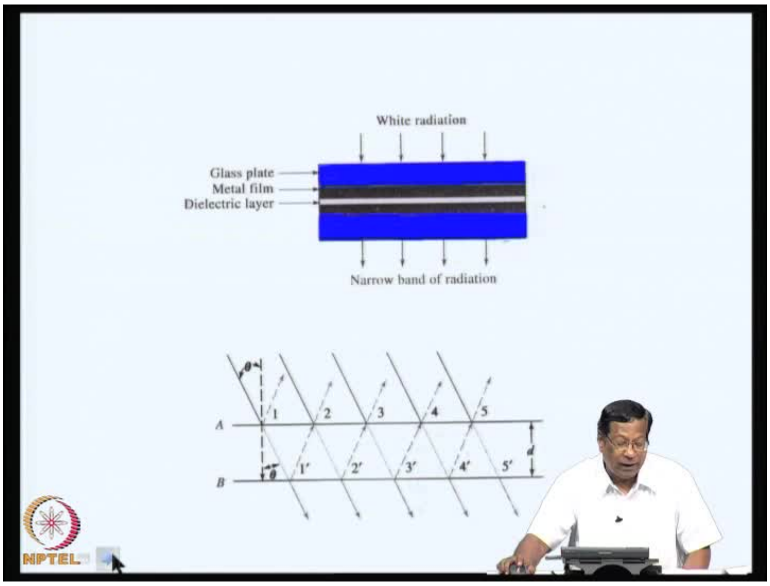

Now, it is essentially the same like what I showed you earlier. These are the glass plates and this is the metal film, this black one. And there is a dielectric layer of calcium fluoride or magnesium fluoride and narrow band of radiation will be coming out of this. And this works on the constructive interference and destructive interference like this, we have discuss these things in the interaction of matter with radiation.

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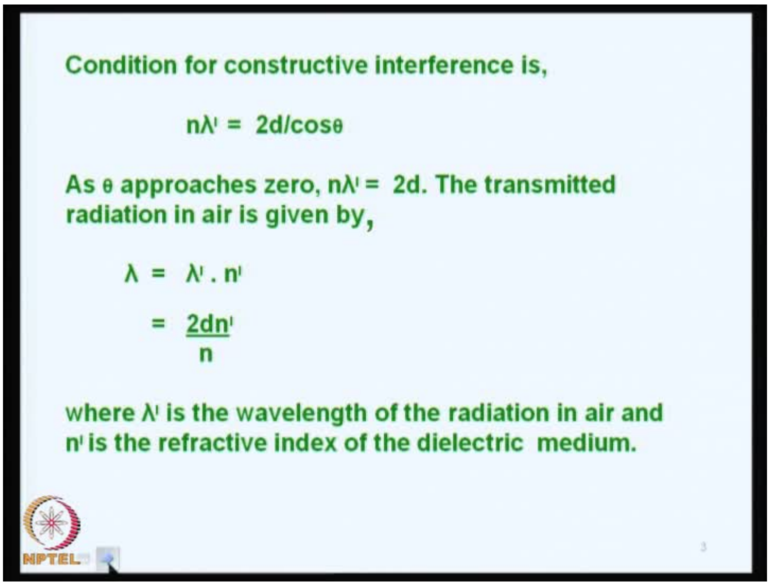

So, these interference filters would give you fairly consistent wavelengths and the condition for constructive interference is given by n lamda is equal to 2d by cosine theta. As theta approaches 0 you would know that this would be 1, so n lamda would be equal to 2d. And the transmitted radiation in the air is given by lamda is equal to lamda dash and n dash, where lamda dash is the wavelength of the radiation in air and n dash is the reflective index of the dielectric medium. So, if you use this expression you would know that approximately it is exactly equal to 2dn dash by n.

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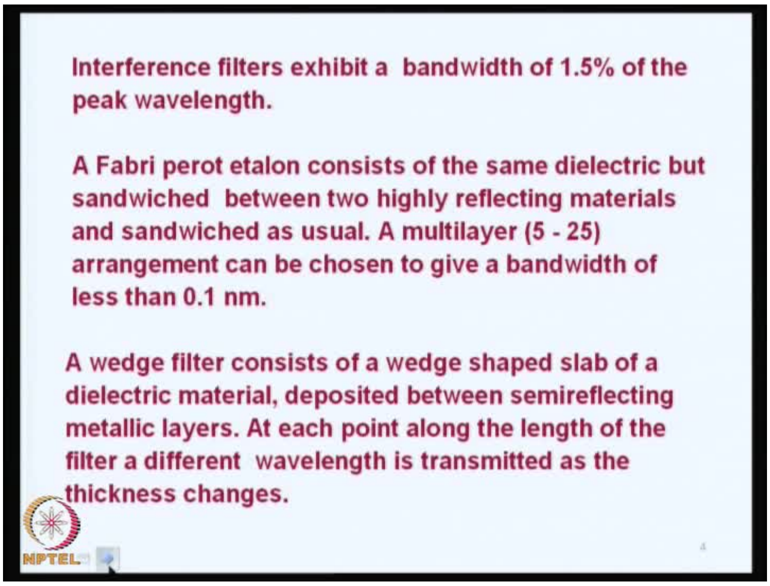

And for every interface it would be showing you this choice. So, interference filters in general exhibit a bandwidth of about 1.5 percentage of the peak wavelength. Another is the filter that we regularly use is the fabric perot etalon that consists of the same dielectric, but sandwiched between two highly reflecting materials and sandwiched as usual. So, a multilayer arrangements can be chosen upto 5 to 25 systems, multilayer sandwiches arrangements, can be chosen to give a bandwidth of less than 0.1 nanometers, that is very good.

Another possibilities you to use a wedge filter consisting of a wedge shaped slab of a dielectric material deposited between semi-reflecting metallic layers. At each point along the length of the filter a different wavelength is transmitted as the thickness changes. So, you can have different wavelengths for your requirement.

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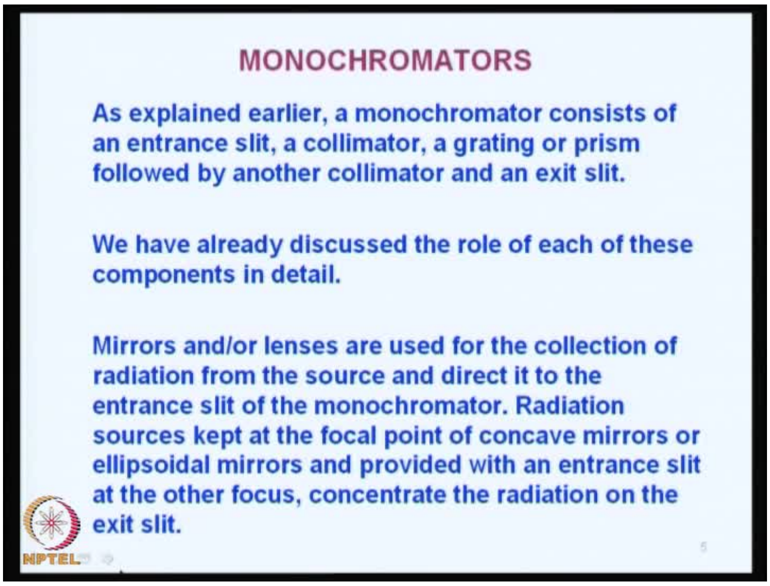

Now, let us discuss about monochromators. Now, what is the monochromators? I have explained to you earlier, a monochromator is an optics system. It consists of an entrance slit, a collimator, a grating or prism followed by another collimator and an exit slit. Instead of grating you can have a filter here, you can have a grating here or you can have a prism there.

We have already discussed the role of these components in details and we will not go again more into that. But what you can do is, I want to tell you that mirrors and lenses are used for the collection of radiation from the source and direct it to the entrance slit of the monochromator, that is all they function of the mirrors. That means, they collect the radiation from the source and direct it. You can put a reflecting mirror at the back or you can put concave lense, convex lens and all those things you can put, but their main function is to direct it to the entrance slit. Only from the entrance slit we will have the radiation passing on to the optics system.

So, radiation sources are kept at the focal point of concave mirrors or ellipsoidal mirrors and provided with an entrance slit at the other focus. So, if these are provided with an entrance slit at the other focus, they concentrate the radiation on the exit slit. So, this is how most of the radiation is collected nd then it has to pass through a slit.

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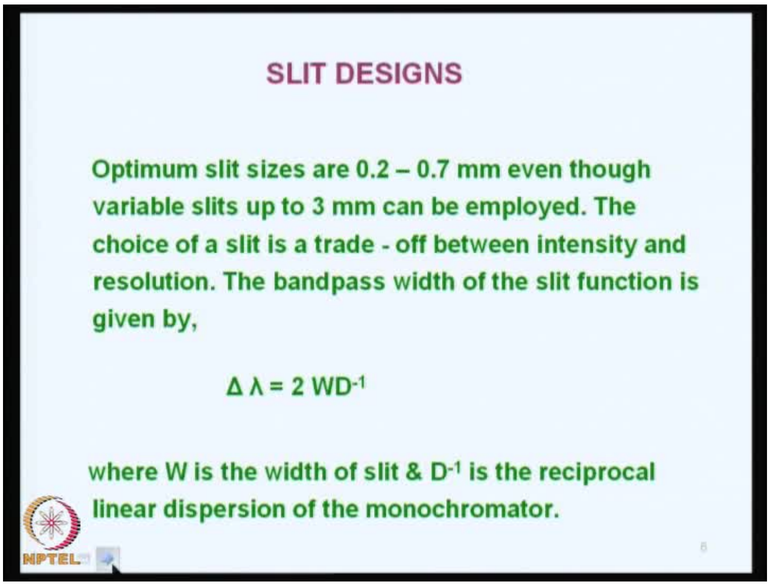

So, the job of the slit is to permit known amount of radiation through a small hole. Basically it is a mechanical operation through which the radiation will be passing through. So, whatever is the size of the slit that will permit radiation to pass through only that, all other radiation will be obstructed. They can be of course, collected in another concave mirror and redirected onto the slit.

So, the optimum slit sizes are of the order of 0.2 to 0.7 millimeter maximum. Even though variables slits are also there nowadays in modern instruments, but they are costly. And you can vary the slits size upto 3 mm, they can be employed.

The choice of a slit is basically a tradeoff between intensity and resolution. Higher the slit the resolution would be less, lower the slit smaller the slit the resolution will be better. So, the bandpass width of the slit function is given by delta lamda is equal to 2 WD inverse, where W is the width of the slit and D inverse is the reciprocal linear dispersion of the monochromator. So, what we are essentially saying is your slit should be as small as possible to get good spectrums, at the same time it should not be too small to lose the intensity.

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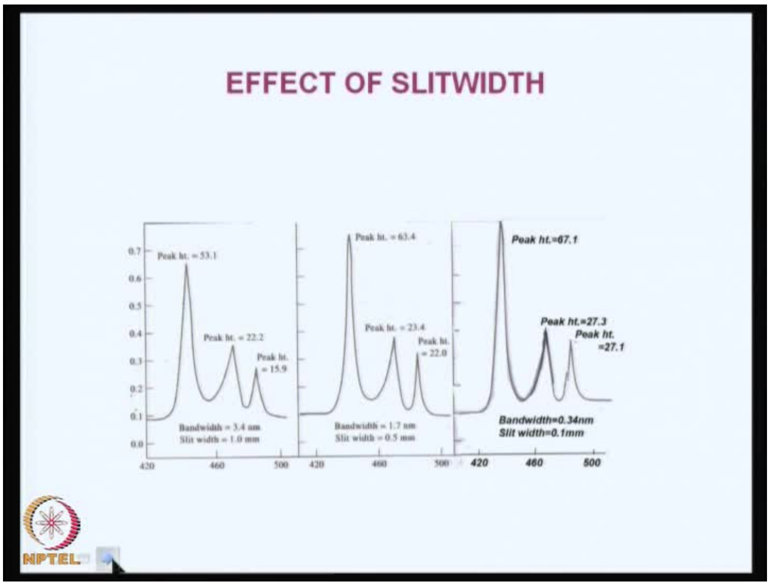

So, you can see the effect of slitwidth here. The first one we have plotted peak height versus wavelength. You can see the peaks are very sharp here. And here the peaks are much better, but peak height you can see as the bandwidth and slit width these 0.5, here it is 1 mm, here it is 0.5 mm, you can see that the peak height here is about 53.1. And as the slit decreases slit width, it goes to 63.4 that is the peak height; that means, the spectrum has become much sharper.

Suppose you reduce it to 0.1 millimeter in the third case like here. Now, the peak height here is 67.1 compared to 63.4 here and 53.1 here. So, from 3.4 to 0.1 mm slit if you change, the peaks will be sharper and the spectrum will be so much purer.

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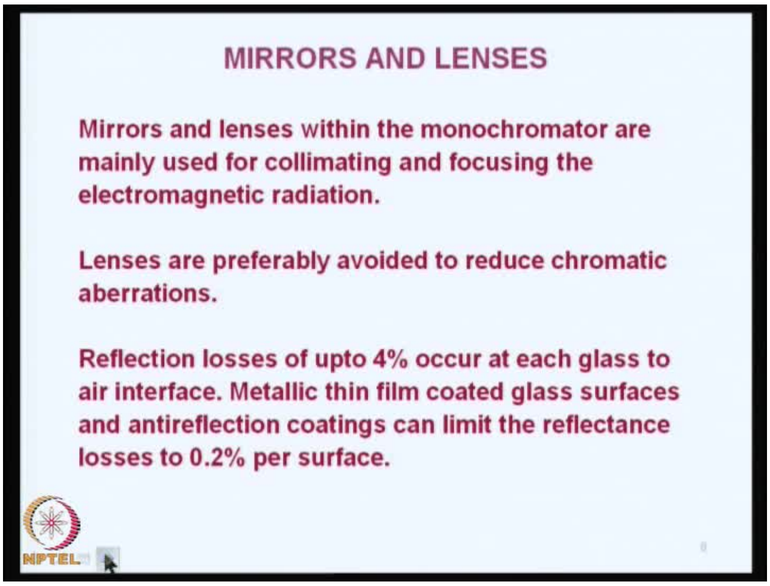

But it is always the trade of between the best slit width and best intensity. So, other aspects of the collimator is mirrors and lenses. And these mirrors and lenses within the momochromator are mainly used for collimating and focusing the electromagnetic radiation. Usually we avoid lenses because they cause chromatic aberrations. And reflection losses also occur upto 4 percent at each glass to air interface. This is bad because the intensity would be so much reduced and the peak would not be so good.

So, metallic thin film coated glass surfaces and antireflection coatings you can use on the lenses and mirrors and they can reflect the reflectance losses, they can reduce 0.2 percent per surface; that means, for every mirror, every lens, every interface the losses would keep on adding by 0.2 percent.

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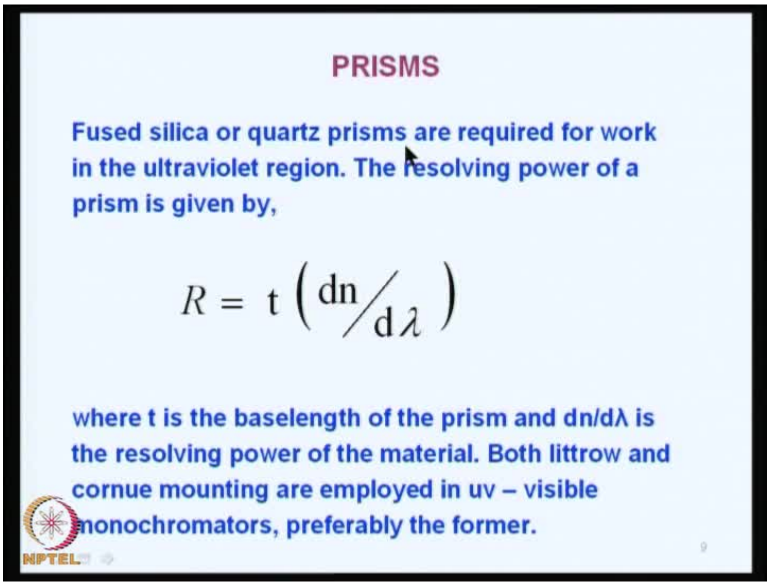

So therefore, for this reason the instruments use minimum number of lenses and mirrors. So, you can use prisms for collecting the wavelength, this also we had discussed earlier. And fused silica or quartz prisms are required for work in the ultraviolet region. The resolving power of a prism is given by this equation, R is equal to 1 t into dn by d lamda, where t is the baselength of the prism and dn by d lamda is the resolving power of the material.

We can use both littrow and cornue mounting and in uv visible region. And preferably the former because the littrow mounting gives you so much of additional space, instrument become compact and the cost will be less.

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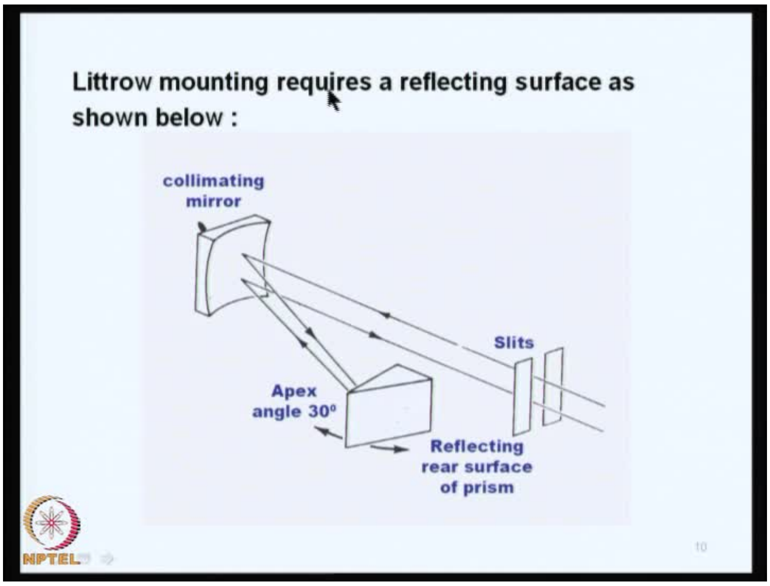

Now, this is a littrow mounting, it requires a reflecting surface like this collimating. So, the slit it will be coming here and then focussed onto the reflecting rear surface of prism and then it passes through it again and then comes back through the entrance slit. This is the arrangement of littrow mounting.

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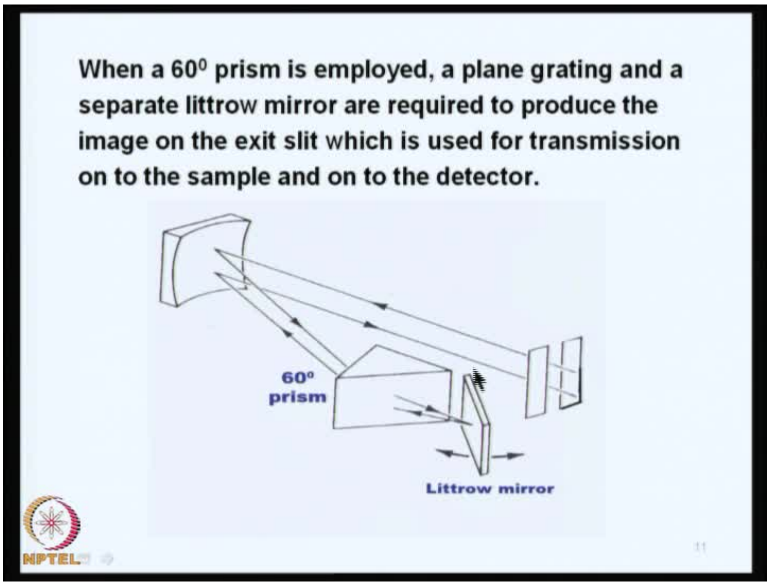

And you can use a prism, 60 degree prism, in carnue mounting. But still here you will see that the littrow mirror has to be on the other site, so the instrument will become slightly bigger, but still you need a littrow mirror to produce the image on the exit slit, which is used for transmission onto the sample and onto the detector. So, you can use a prism with littrow or 60 degree carnue mounting.

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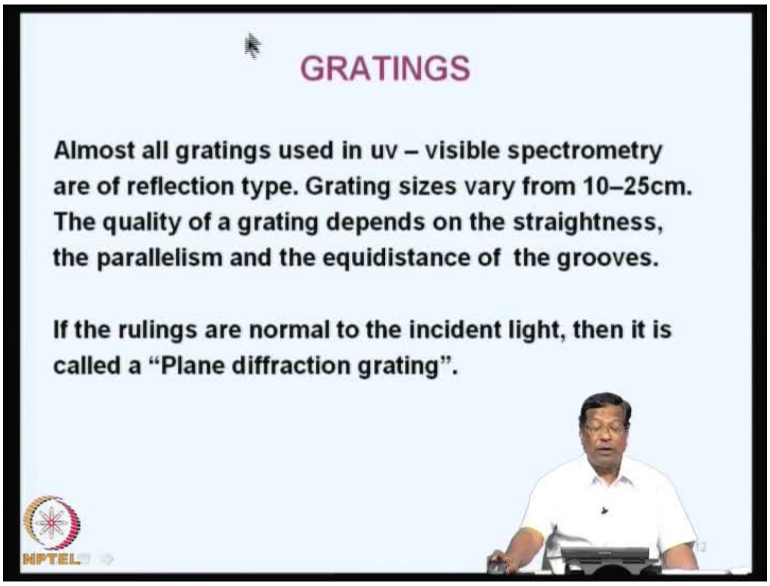

Now, instead of all these things, we can have gratings. Gratings, as we have already discussed they give beautiful small sharp peaks and almost all gratings used in uv visible spectrometry are of reflection type. So, grating sizes you will be surprised to know that they vary from 10 to 25 centimeters. The quality of a grating actually depends upon the straightness, the parallelism and the equidistance of the grooves.

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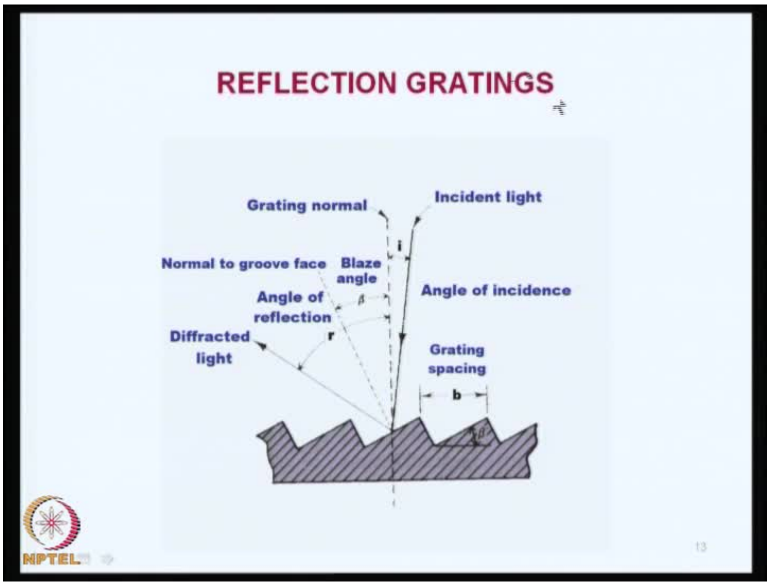

If the rulings are normal to the incident light, then we call it a plane diffraction grating; that means, you draw the lines perpendicular like this and there would be upto 30 thousand lines you can draw per centimeter, you can imagine that it will how much reflecting surface it gives. And as you rotate the grating the wavelength will be selected, this is the general design of a grating, reflection a grating. Here you can see that this is the grating normal, this is the angle of reflection and this is a blaze angle and this is directed light, incident light is there, it falls on the broad surface and b is the grating spacing; that means, this spacing in a format of this spacing makes all the difference in the quality of the grating.

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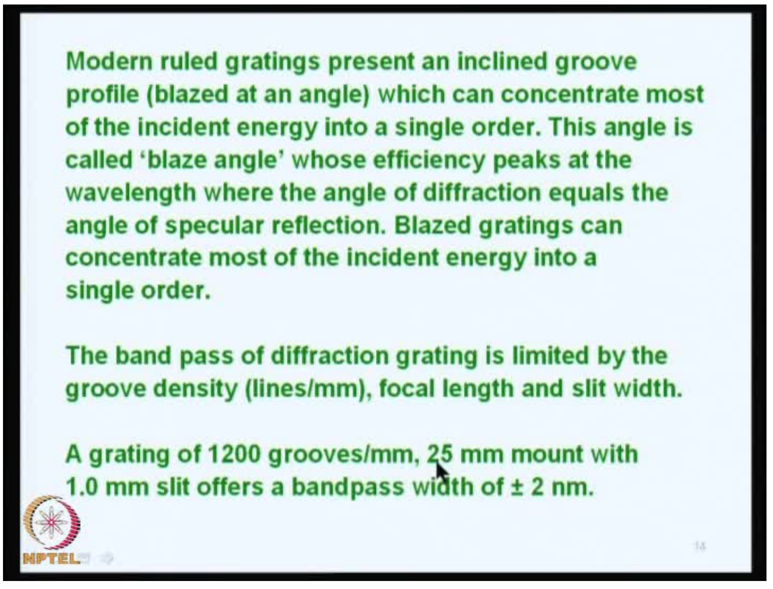

Now, modern ruled gratings present an inclined groove, not exactly straight gratings, but in an inclined way, so that the broad surface will be presented at the incident angle. So, this is known as blazing. So, this modern ruled gratings are blazed at an angle which can concentrate most of the incident energy into a single order. This angle is called as blaze angle, whose efficiency peaks at the wavelength where the angle of diffraction equals the angle of specular reflection. So, blazed gratings can concentrate most of the incident energy into a single order, this is the advantage of a blazed grating.

Now, a band pass of a diffraction grating is limited basically by the groove density. its focal length and slit width. So, a grating of about 1200 grooves per mm, this is what usually used in the gratings. And 1200 grooves, 25 mm mount with 1 mm slit offers a bandpass width of plus or minus 2 nanometers. That is you can choose a wavelength of about 430, you would have wavelength coming containing 428 and 432. So, 430 would be highest maximum intensity whereas, 428 would be having lowest intensity and 432 also would be having lower intensity.

Now, you can have gratings of about 1800 grooves per mm, they are also used extensively in most of the instruments and upto 3000 and also have been used. But in most of the spectrophotometers you come across, the grating would be of about 1200 or 1800 maximum, you will not come across higher rules ruled gratings than that. Because the spectrum, you do not need still higher resolution in molecular spectra because most of the peaks are broad.

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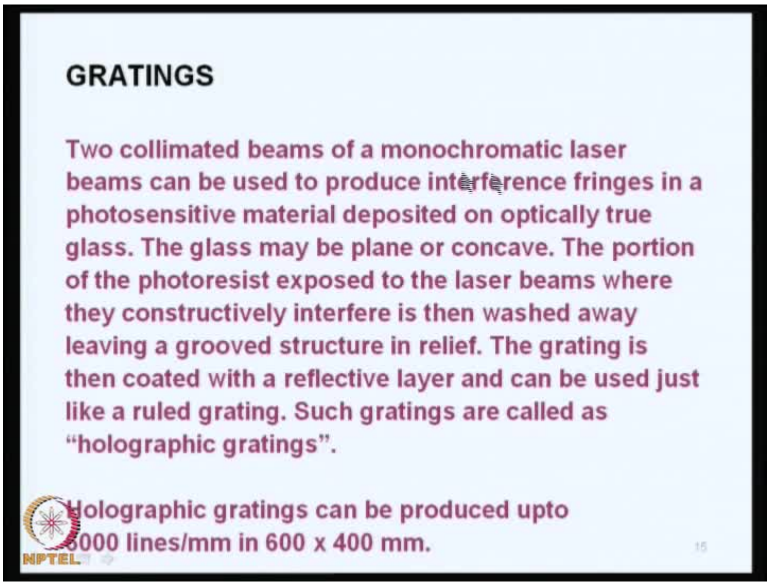

Now, we continue our discussion. What is basically a grating is two collimated beams of monochromator, monochromatic laser beams can be used to produce interference fringes in a photosensitive material deposited on optically true glass. So, how do we do a grating? We take two collimated laser beams and then use them to project them onto the glass coated with a photosensitive material. And when the photosensitive material falls on the grating, it reacts it will be exposed. The glass may be plane or concave, but the portion of the photoresist exposed to the laser beams where they constructively interfere is then washed away and then leaving a grooved structure in the relief. That means, in the parent structure whatever is the grating of photoresist material is there, as the laser passes it cuts and eats away. So, this is washed away leaving an unreacted photoresist material, which is nothing but the grating. So, this grating is then coated with anti-reflective layer and can be used, basically first reflective layer, and it can be used like a ruled grating. Such gratings are called as holographic gratings. So, these holographic gratings are basically manufactured using a two collimated laser beams to produce interference fringes.

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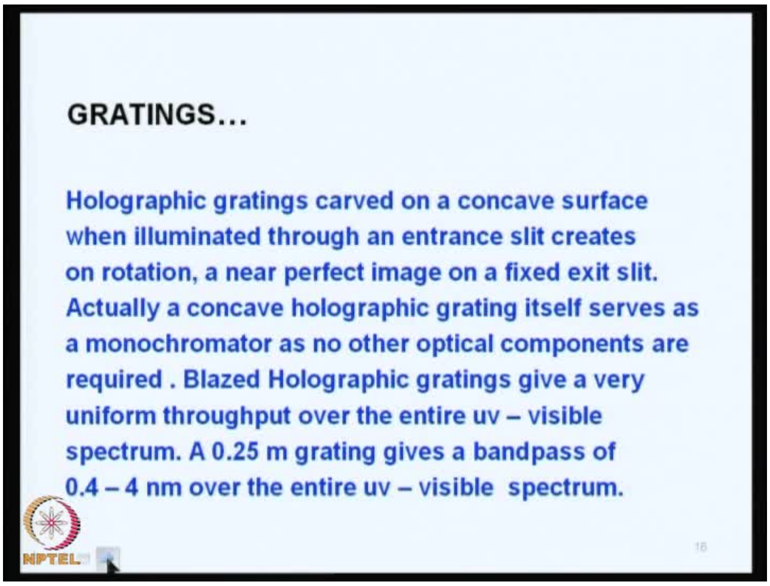

So, holographic gratings can be produced upto 6000 lines per mm in 600 by 400 mm mirrors. And you can have wonderful holographic gratings. You would have seen many of such gratings in day today life also with lamps. When you look at some lamps this way or this way you rotate the lamp, you will see the picture of the bird in different colors etcetera, they are all made by holographic gratings.

Now, holographic gratings carved on a concave surface when illuminated through an entrance slit of course, you all the time you will have to illuminate it through the entrance slit only. On rotation what it does? It gives a near perfect image on a fixed exit slit. Actually, a concave holographic grating itself serves as a monochromator, you do not need anything else, especially if you are using a concave holographic gratings because the grating is pure there are no distortions and then there will be perfect concentration of the radiation that falls and reflected and it will we collected and at the focal point of a concave grating at the exit slit. So, that itself serves as a monochromator because you do not need any other optical components. So, the cost will be less and spectrum what your radiation what you get are so much purer. So, blazed holographic gratings give a very uniform throughput over the entire uv visible spectrum unlike prisms. Prisms, the dispersion is not linear whereas, in blazed holographic gratings you get linear spectrum.

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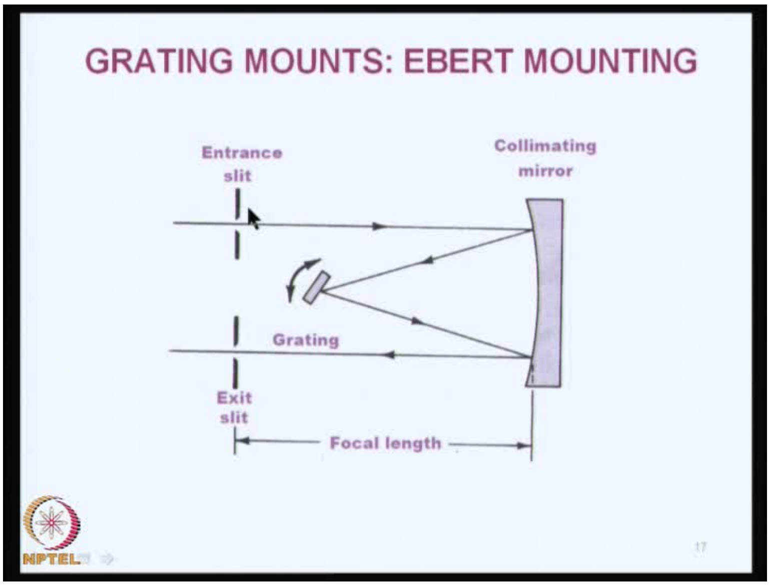

So, a 0.25 millimeter grating gives a band pass width of 0.4 to 4 nanometers over the entire uv visible spectrum. This is the beauty of blazed holographic gratings. Now, it is not just enough if you use gratings, you have to put the gratings in a specific position in the instrument where the entire slit has to, where entrance slits and other things are there. So, the positions of this entrance slit and this collimating mirror and grating all these things have to be fixed in such a way that the image of this entrance slit falls on the exit slit.

So, this positioning of these and positioning of the grating, positioning of the mirror, positioning of the entrance slit and exit slit they are all called as mounts. Different kinds of arrangement can be made to collect the radiation. Here I am showing you a grating mount known as Ebert mounting. In Ebert mounting, what we have? We have an entrance slit here, a collimating mirror, this you can see that it is a concave mirror, and the radiation comes here it falls on the grating gets reflected, falls on the grating and gets reflected again, falls on the same grating at the other side and gets reflected onto through the exit slit. So, you can see that both these things, entrance and exit slits, are at the focal length of this mirror. This is known as Ebert mounting. Here we can see that only one mirror serves for both incident as well as exit radiations.

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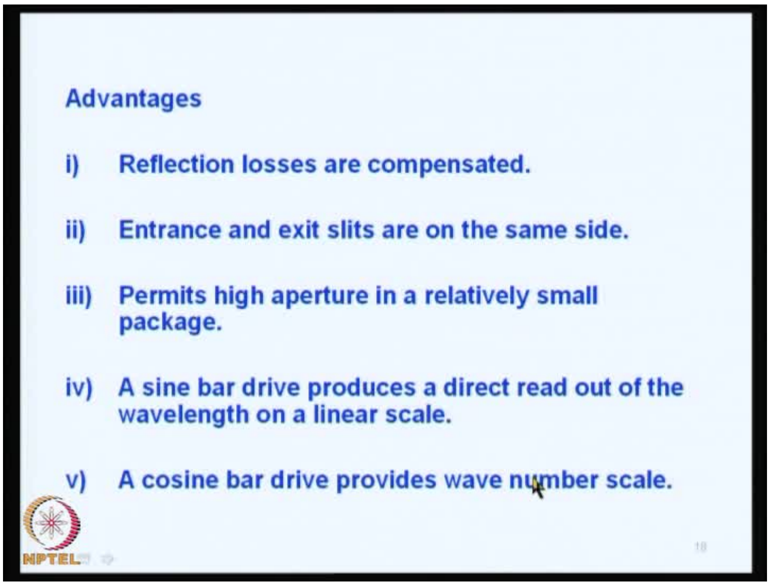

Now, you can see the advantages. Only one mirror we are using therefore, reflection losses are less. Entrance and exit slits are on the same side; that means, this will result in the compact instrument if you use Ebert mounting. And then this permits high aperture in a relatively small package. So, a sine bar drive produces a direct read out of the wavelength on a linear scale. So, as I have told you the grating you will get always linear scale, so there wont be much reflection losses. A cosine bar drive provides the wave number scale, this would be useful more in infrared spectrum rather than the uv visible spectrum, where we use the wave numbers.

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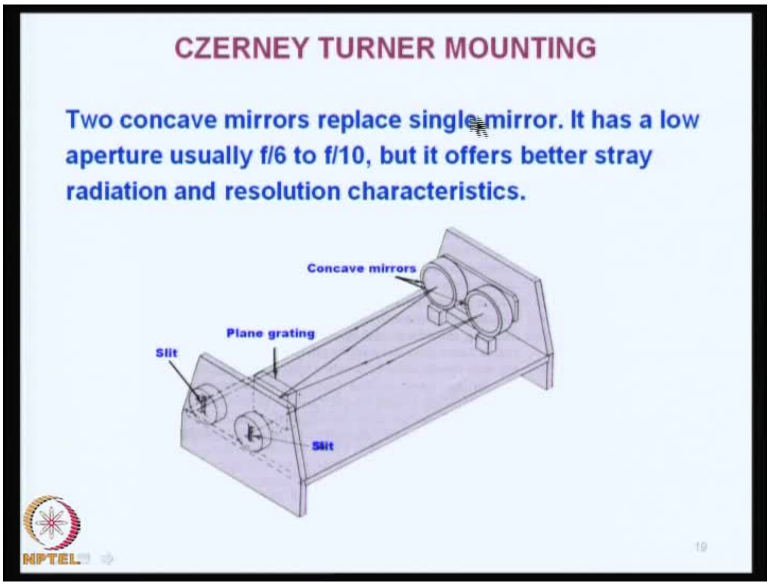

Now, another grating mounting is very popular in most of the instruments. So, here what you do is, we are using two concave mirrors replacing the single mirror in the Ebert mounting. So, I have two concave, one here and another is here, and it has low aperture usually of f 6 to f 10, but it offers better stray radiation control and better resolution characteristics. Here you can see that this is one slit, this is another slit, this is the plane grating, and the radiation comes here, it falls on the plane grating and then it falls on another mirror, concave mirror, and then comes out through the exits slits. So, this is another arrangement which is very popular in most of the spectrophotometers and even atomic absorption spectrometers.

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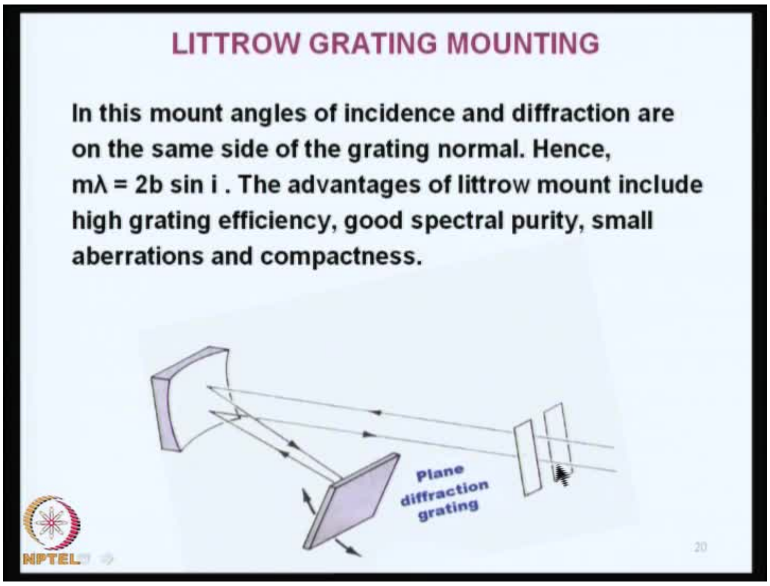

Now, you can mount the grating in a littrow fashion. In this mount what happens is the angles of incidence and diffraction are on the same side. See the radiation is coming like this, falling here, this is a plane diffraction grating, comes out through the same exit slit. So, only thing is the height would be different. So, the advantages of littrow mount obviously include high grating efficiency, good spectral purity, small aberrations and compactness and then aberration and compactness, that is the basically the advantage.

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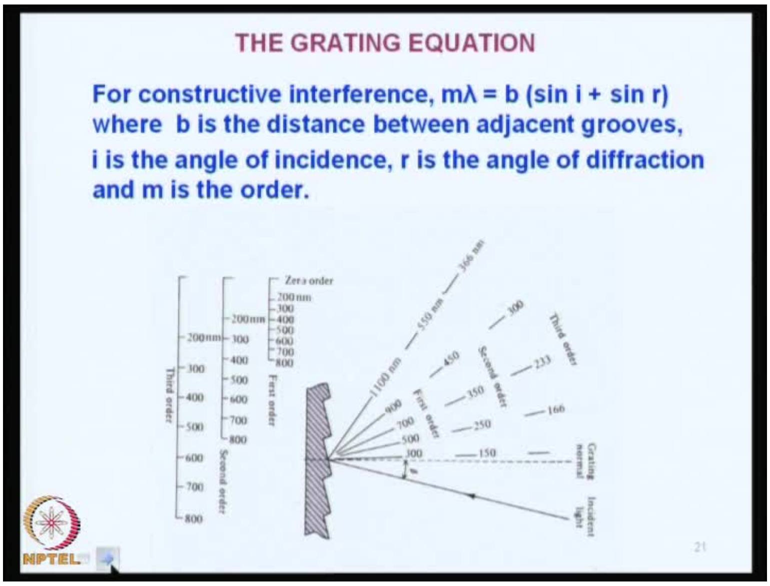

You can see that in the grating the basic equation m lamda is equal to b sin i plus sin r, where b is the distance between the adjacent grooves, i is the angle of incident and r is the angle of diffraction and m is the order. As I have told you the grating provides different orders of the radiation. For example, here you can see the first order scale is very linear here, 800, 700, 600, 500 etcetera, this is known is zero order. And then we have another second order coming so 200, 400, 300, 400 upto 800. Third order also is there, that also covers the same range. But the first order intensity of the first order output would be much higher in this case compared to this second order compared to third order. We can see that we can use any number of orders up to 10 also.

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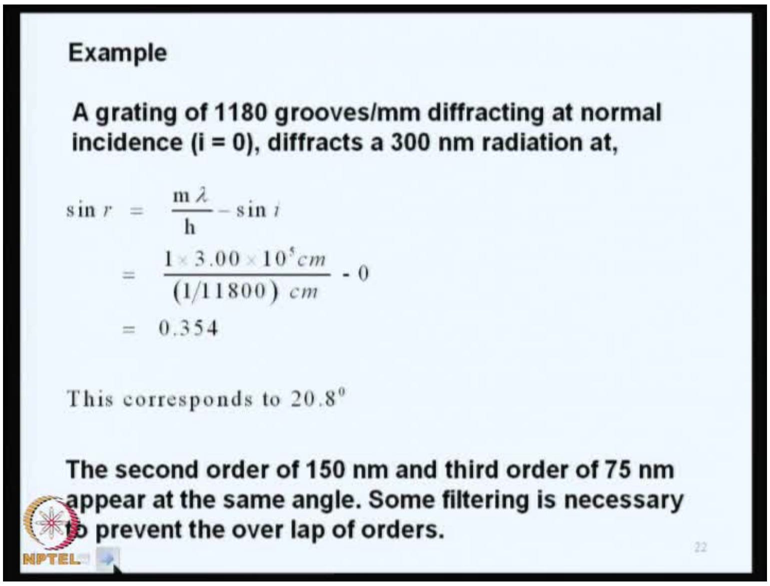

Now, take a look at this example. That is a grating of 1180 grooves per mm diffracting at normal incidence diffracts a 300 nanometer radiation. That means, we have taken a grating which is having 1180 grooves and you are directing a radiation from the source which contains 300 nanometer radiation. Now, what happens? If you use this equation, sin r is equal to m lamda by h minus sin i. So, you put the values here, you will get 0.354; that means, this corresponds to 20.8 degrees. That means, if you use a grating you use three, term the grating to 20.8 degrees, what you get would be 300 nanometer radiations in the first order. It will also be associated with the second order at half the intensity or still lower intensity. But the second order wavelength would correspond to 150, half of this 300 to and 150. So, what would be the third order? The third order would have 75 nanometers. They all appear at the same angle therefore, some filtering is necessary even after you use the grating to avoid the second order and third order wavelengths getting mixed up along with the basic wave length; that is, 300 nanometers. I have given you this example just show you that different kinds of spectrophotometers have different orders getting mixed up and it is a essential to use some sort of a filter to avoid second order, third order because they do not actually represent any constructive spectra, so they are all waste any way. So, it is better to avoid them to get a purer spectra.

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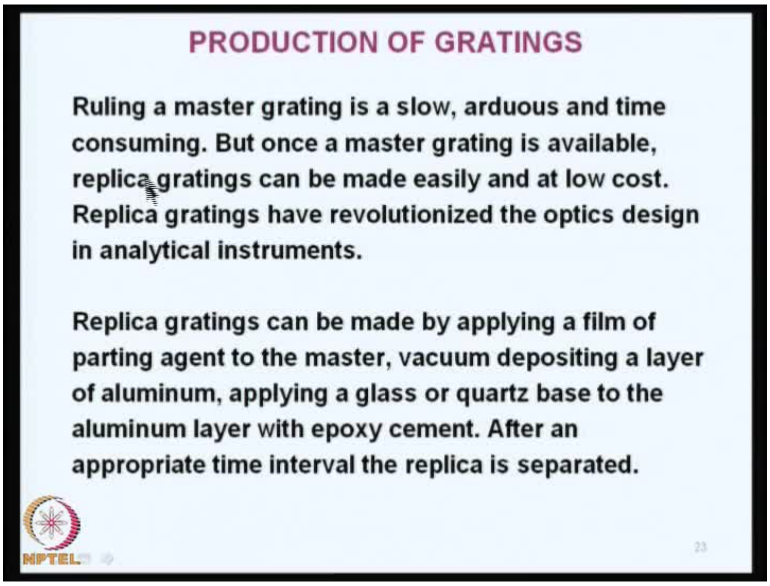

So, how do you produce gratings? Master gratings are usually produced by ruling a master grating. We have to take a small slab of glass and then you have to take diamond tipped tool and keep on ruling the gratings. And ruling a master grating is a very slow arduous and time consuming. But once a master grating is available, replica gratings can be made easily and at low cost. Once these replica grating have actually revolutionized the optics design in analytical instruments because replica gratings are very cheap to produce. And how do you make them? You take the master grating, apply a thin film of parting agent to the master grating and then vacuum deposit a layer of aluminum. And this aluminum applying on a glass or quartz base to the aluminum layer with epoxy cement. And after an appropriate time you just remove the top portion. First you have a grating here and then you are putting aluminum and then parting reagent and vacuum depositing a layer of aluminum, applying glass are quartz base, remove it and apply quartz plate and then remove it, so the master grating remains at the bottom, its reflection comes on the top which is again a grating. So, after an appropriate time interval you can just remove the grating and you have replica grating reading.

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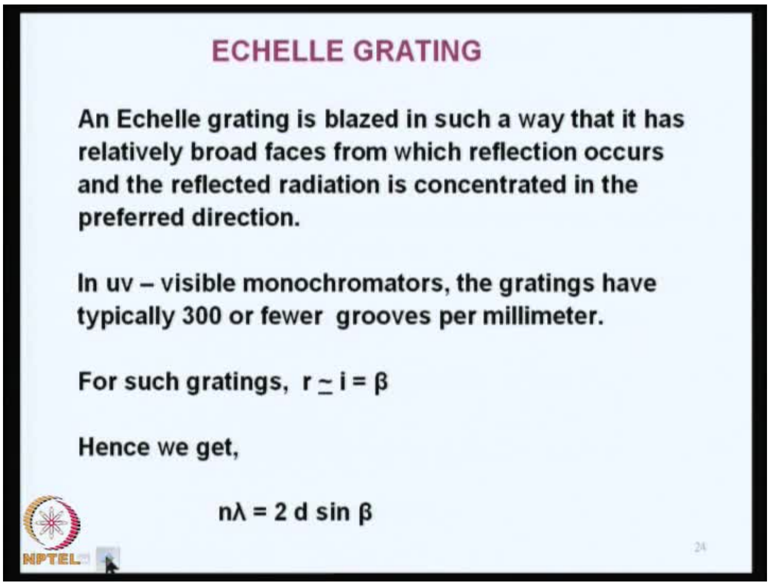

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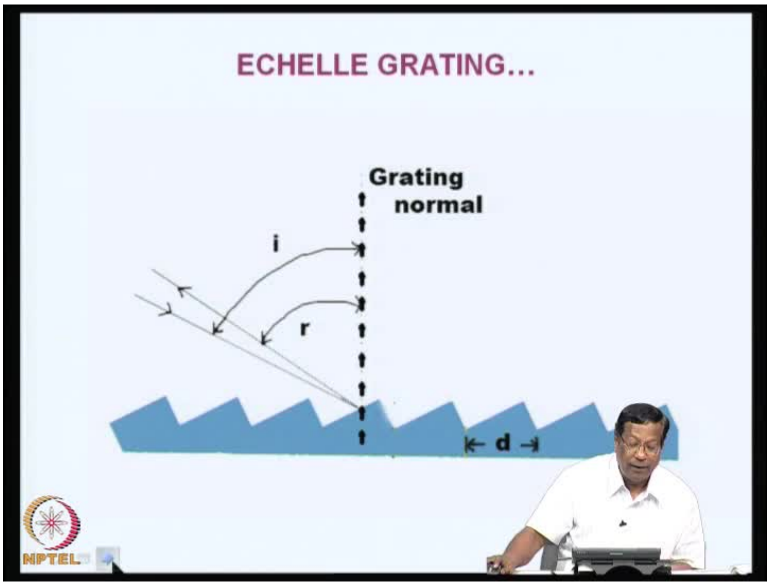

So, another thing that you come across, that is a grating, is known as is echelle grating. So, an echelle grating is slightly different from holographic gratings. But it is blazed in such a way that it has relatively broad surfaces from which reflection occurs and the reflected radiation is concentrated in the preferred direction. Basically if you take a look at a grating, you have a broad face here and a small face here. This is echelle grating.

So, when you make the radiation fall on the broader grating, broader face, you will get a better a reflection. And this is what is taken advantage of in a spectrophotometers. And the reflected radiation is basically concentrated in the preferred direction. In uv visible spectrophotometers monochromators, the gratings have typically about 300 or fewer grooves per millimeter. Compare this with 1200 or 1800 lines grooves per centimeter and that is not very good, but echelle gratings give you a better output. For such gratings you can say that angle of reflection is almost equal in to angle of incidence. So, if you put this in the equation, n lamda is equal to 2d sin beta. So, what does it mean? It means basically is the spectrum would be so much purer.

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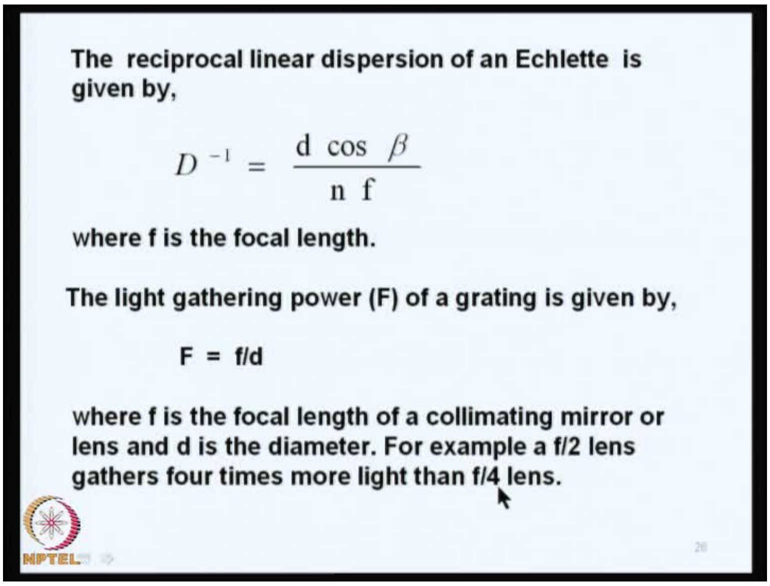

The reciprocal linear dispersion of an echlette is actually given by d inverse would be equivalent to d cosine beta over n into f, where m f is the focal length. So, the light gathering power f of a grating is given by f by d, where f is the focal length of a collimating mirror or lens and d is the diameter. For example, a f by 2 lens gathers 4 times more light than f by 4 lens. So, it is preferable to use as small as possible instead of f by 2 f by 1 like that and compared to f by 4 because you would be collecting more radiation by using f by 2.

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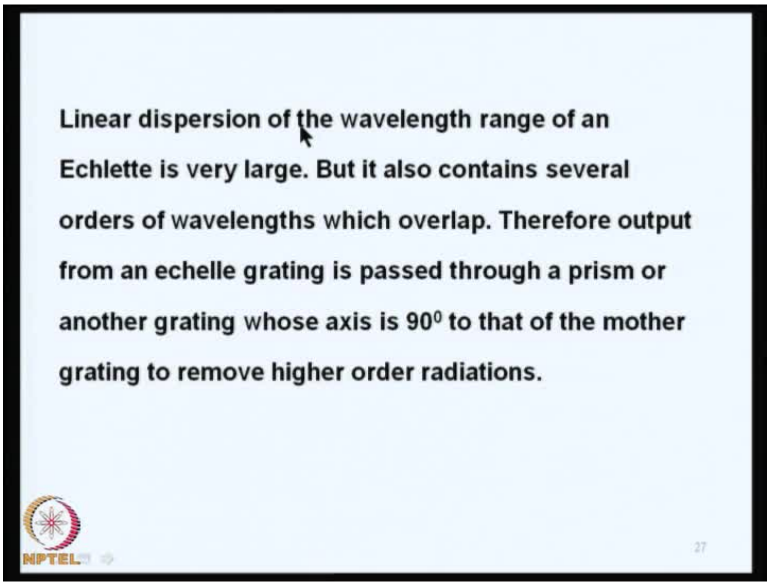

So, if you take a look at the linear dispersion of the wavelength range of an echlette you can see that it is very large. But it also contains several orders of wavelengths which overlap and we do not need the several orders. So, what do you do? When we do not need it, we have to put some filter as I explained earlier. So, output from an echelle grating is always passed through a prism or another grating, whose axis is 90 degrees to that of the mother grating to remove higher order radiations. So, if you use echelle grating the complexities of the optical design are much more compared to other designs. But the purity of this spectral line that is also very important. So, if you want a very good spectrophotometer, very good spectrum, you have to pay for that and that costs more.

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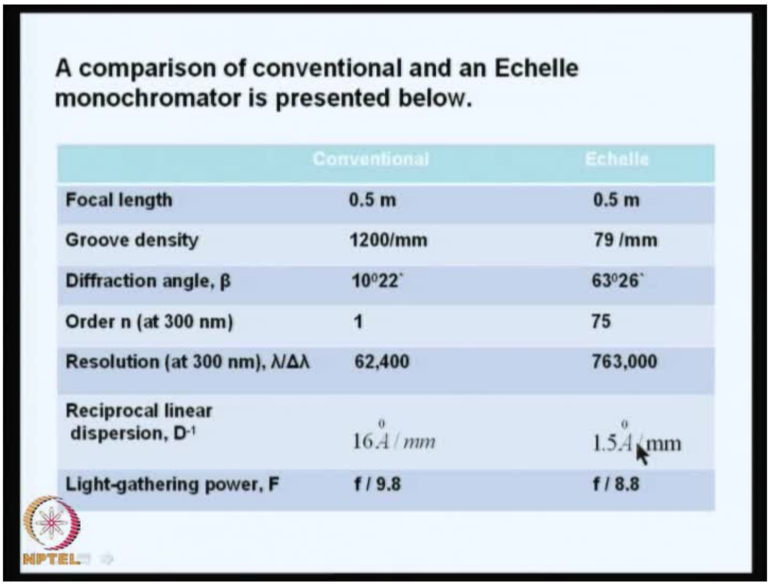

	Conventional	Echelle
Focal length	0.5 m	0.5 m
Groove density	1200/mm	79 /mm
Diffraction angle, β	$10^0 22'$	$63^0 26'$
Order n (at 300 nm)	1	75
Resolution (at 300 nm), $\lambda/\Delta\lambda$	62,400	763,000
Reciprocal linear dispersion, D^{-1}	$16\overset{0}{A}/mm$	$1.5\overset{0}{A}/mm$
Light-gathering power, F	f / 9.8	f / 8.8

Now, I can show you here a comparison of the conventional and echelle monochromator. Here you can see that focal length is 0.5 m and echelle also has a focal length of 0.5 m. So, the groove density in a conventional grating is 1200 whereas, in a echelle it is only 79 mm, 79 lines per millimeter.

Diffraction angle would be 10 degrees 22 minutes, and here it is 63. You can see that almost 5 to 6 times the diffraction angle beta varies. So, the order here it is only 1 in conventional whereas, in echelle you can collect radiation upto 75 orders, that is substantial. And all the orders meet to be separated, but the separation of the order are very simple mechanically are in the equipment design.

So, the resolution 62400 compared to 763000 and reciprocal linear dispersion were this 16 armstrong per mm; that means, if you rotate the radiation, you would get 16 armstrong per millimeter of rotation. Here you get 1.5 armstrong, that is a fantastic improvement over the spectral purity. And you can also see here that the light gathering power F is f by 9.8, here it is only 8.8.

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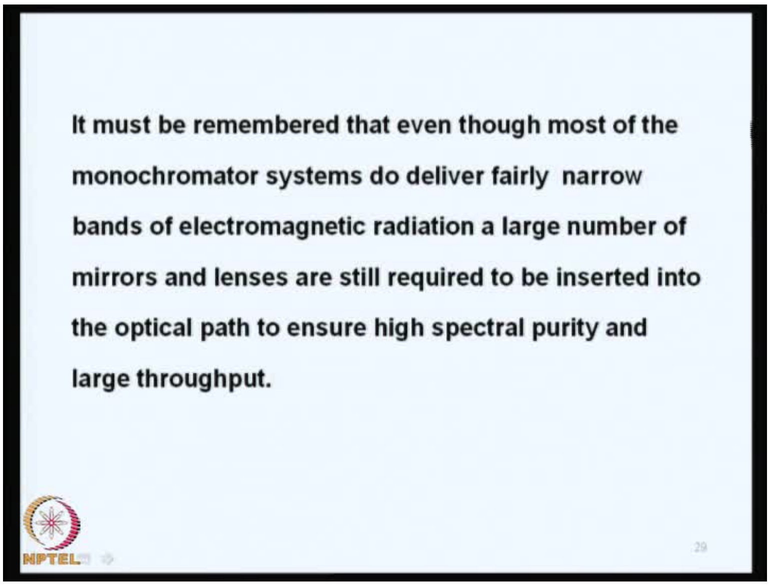

Obviously you can appreciate the advantages of echelle, I need not say more. So, whenever you have to buy an equipment, a spectrophotometer, you have to remember all these things and find out whether it is a prism instrument, whether it is a grating instrument or whether it is a filter instrument. And if it is grating, whether it is plane grating or concave grating or whether it is a blazed holographic grating has been use or simply echelle grating. All these things you like to consider depending upon the quality of the work that you intend to do or the cost considerations. So, obviously the echelle spectrophotometers would need additional optics and that would cost definitely slightly more than the all remains. So, it must be remembered that even though most of the monochromators systems do deliver fairly narrow bands of electromagnetic radiation, a large number of mirrors and lenses are still required to be inserted into the optical path to ensure high spectral purity and large throughput. This is the some sort of a this slide, actually it is a some sort of a summation of our discussion on the optics collimation. And what we are still saying is you cannot really completely avoid the use of lenses and mirror in spectrophotometers, obviously not. But they are still required to be inserted into the optical path to ensure high spectral purity.

For example, if you use echelle gratings, you definitely need prisms mirrors and other things etcetera, you cannot avoid them. Even though technically we says that collimating gratings by themselves are monochromators, but still to avoid stary light, to avoid other interferences, aberrations all these things need to be taken care of in a spectrophotometer and they need to use them in conjunctions with mirrors, lenses etcetera. You cannot avoid them, but it is good to have theses things to get a better this thing.

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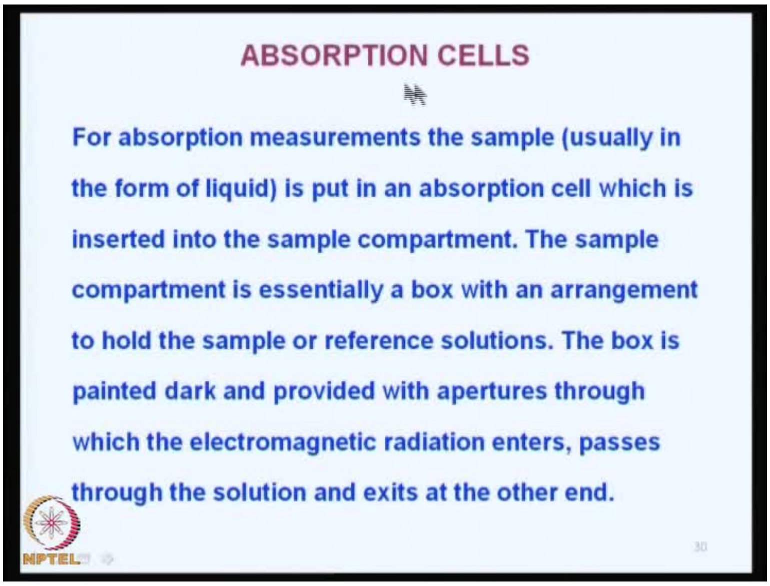

Now, we move on to the other aspect of the spectrophotometric instrumentation, that is the absorption cells. So, absorption measurements, what we do is, we take the sample in the form of liquid and it is put in an absorption cell which is inserted into the sample compartment. What is an absorption cell? An absorption cell is a…

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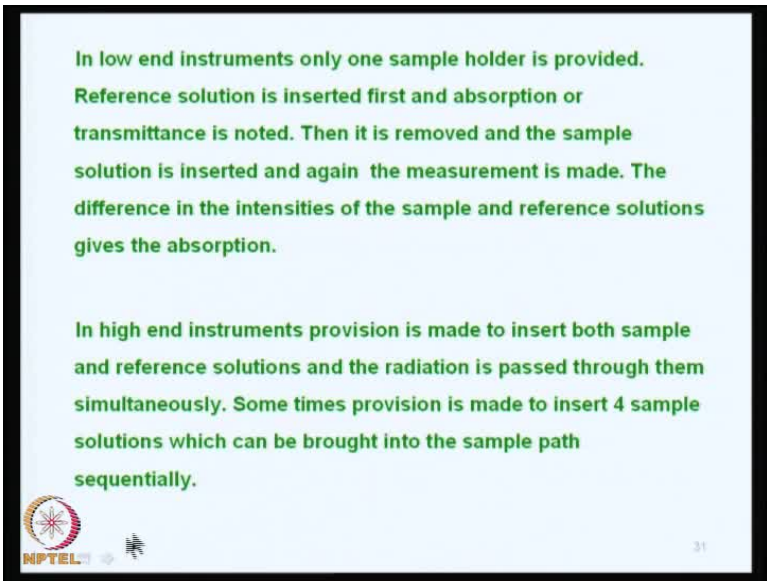

So, in the low cost, low end instruments only one sample holder is provided it; that means, you have to put the reference and take the absorbents measurement and then remove it from the system, put your sample again and again take the measurement like that only one sample holder; that means, it can hold only one sample and reference solution is inserted first and absorption or transmittance is noted. Then you have to remove it, put another sample, again make the measurement and the difference in the intensities of the sample and reference solution gives the absorption.

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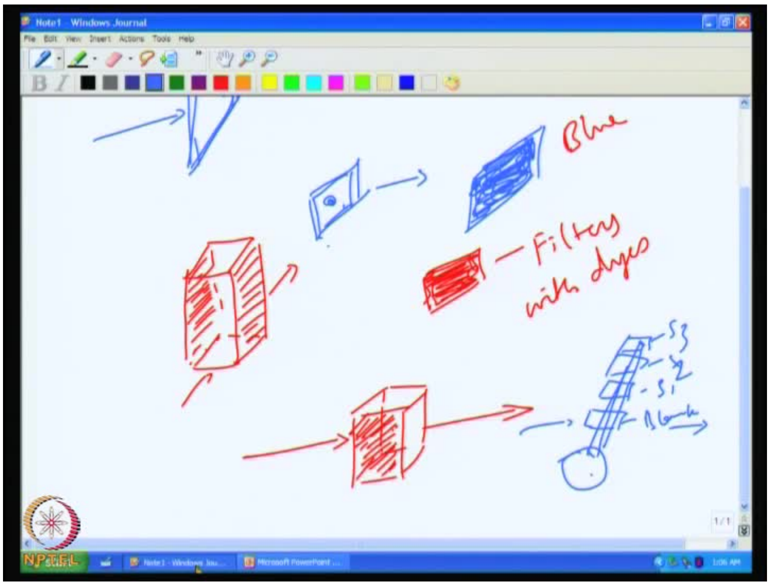

See, in high end instruments what they do is they put both samples, sample and reference solutions and the radiation is passed through them simultaneously. Sometimes provision is made to insert four sample solutions, which can be brought into play as and when we need.

For example, you can have a rod made like this and then slits here 1, 2, 3, 4 This can be blank, this could be sample 1, this could be sample 2, this could be sample 3 and then there is a knob here, you can pull this knob one by one to bring the solution, blank sample etcetera to pass the radiation. If you pull, first it will cover the blank, as you pull it s 1 will sliding into the position of the blank. And then s 2 if you pull it again, s 2 will come, s 3 will come etcetera, like that you can accommodate up to four units.

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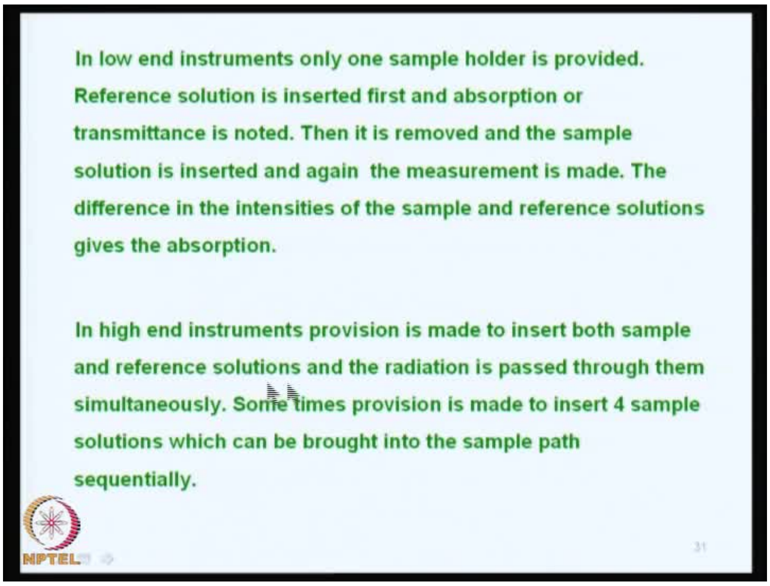

Now, in high end instrument that is what I was trying to tell you, to insert both samples and reference, radiation is passed through. For this you have to make the radiation, split the incoming radiation into two so that any changes in the incident radiation affects the sample and blank simultaneously giving you a purer range absorbents.

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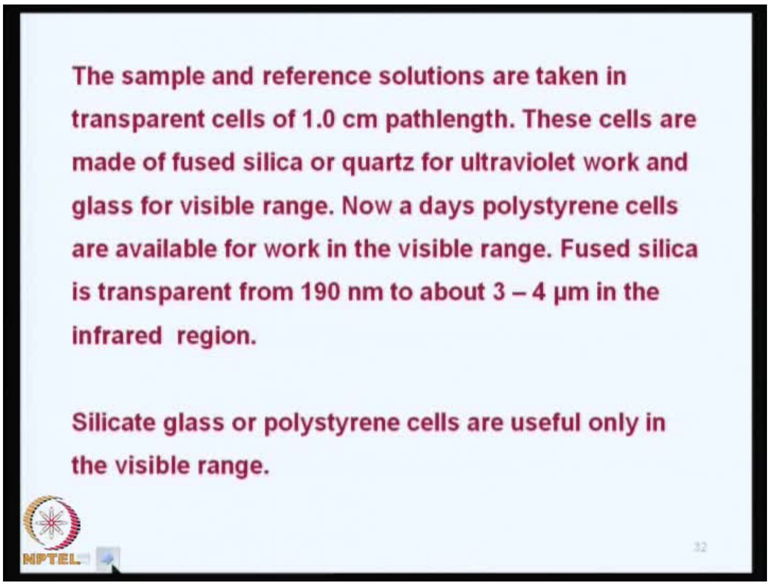

So, the sample and reference solutions are taken in transparent cells of 1 centimeter path length. These cells are made up of fused silica or quartz for ultraviolet work and glass for visible range. Suppose you want to do ultraviolet, work that is work in the range of 180 to 350 nanometers, then you need a silica cell quartz. And for 350 to 800, you would need a glass cells. Now, you can use a quartz cells also to use from 190 to 900 nanometers whereas, fused silica cells are transparent from 190 to 3 to 4 micrometer that is in the infrared region also, near infrared. Silicate glass or polystyrene cells are available in the market which are much cheaper, they cost you hardly 50 paise or 1 rupee per this thing, they can be used for only in the visible range. But since they are very cheap they are of use and throw type, they cannot be cleaned. And these polystyrene cells are also easy to major in visible region range only, they are not useful for ultra violet my range, that is 190 to 350 you cannot use them.

So, you will have to make sure that both the reflecting surfaces are very clean every time you want to usually spectrophotometer because the heart of operation is there in the measurement and the sample cell has to be made, has to absolute and scrupulously clean. And you will have to wash them with nitric acid and sometimes chromic acid, sometimes with acetone and distilled water etcetera. There is a separate procedure for that and to remove the traces of the previous sample. And the polished surfaces of the absorption cells, you should not touch them with your fingers, they will leave finger print marks and they will have you wrong result. So, one as to be extremely careful if you want to use these quartz cells. The quartz cells cost approximately about 2000 to 3000 rupees in India now. And polystyrene cells will cost you about only about 3 or 4 rupees whereas, a glass cell, 1 centimeter cell, would cost about 200 300 rupees range. So, for visible work and for demonstration purposes it is better to use glass or polystyrene and for very accurate work in uv and visible region one can for the quartz cells.

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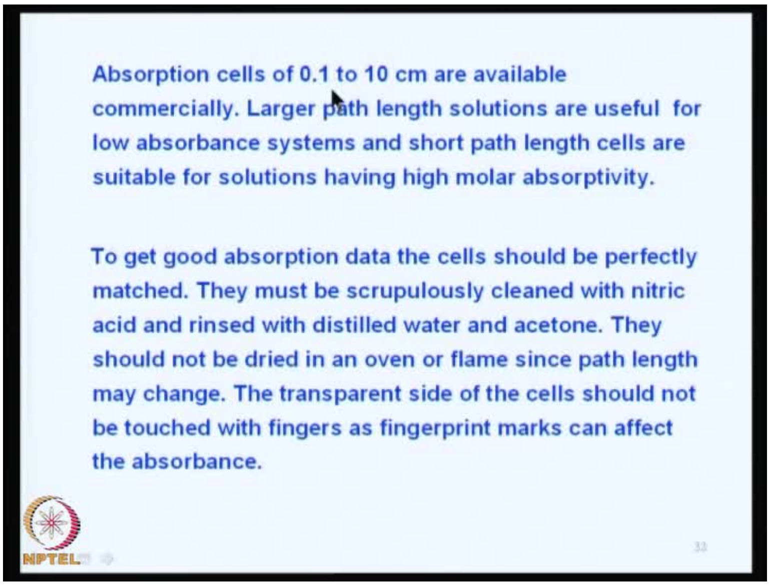

So, absorption cells of the path length is also another important factor in the choice of absorption cells. 0.1 to 10 centimeters cells are available and larger path length solutions are useful for very low absorbance systems. If the absorption very less, we can use 2 centimeter cells, 3 centimeter cells, 5 centimeter, 3 are not available, but 5 centimeters and 10 centimeters path length cells are available in the market. And for high absorption substances, we can go for 0.5 centimeters cells path length or 0.2 centimeters. Even 0.1 centimeter cells are available and of course such thing are characterized by high molar absorptivity. To get good absorption data, the cells should be perfectly matched; that means, there should not be any difference in the cells, that is reference as well as the sample.

So, they must be perfectly match, they must be scrupulously cleaned with nitric acid, rinsed with distilled water and acetone and etcetera. They should not be dried in an oven or flame since path length may change. As a rule you should never dry the absorption cells, quartz cells or glass cells for that matter and plastic also. And polystyrene you should definitely not be drying them in an oven. The transparent side of the cells should not be touched with fingers, as fingerprint marks can affect the absorbance, this I have already explained to you. And that is all I can tell you about the absorption, just that nothing much to tell, but once you start using them, you would really feel that you will learn all this things automatically.

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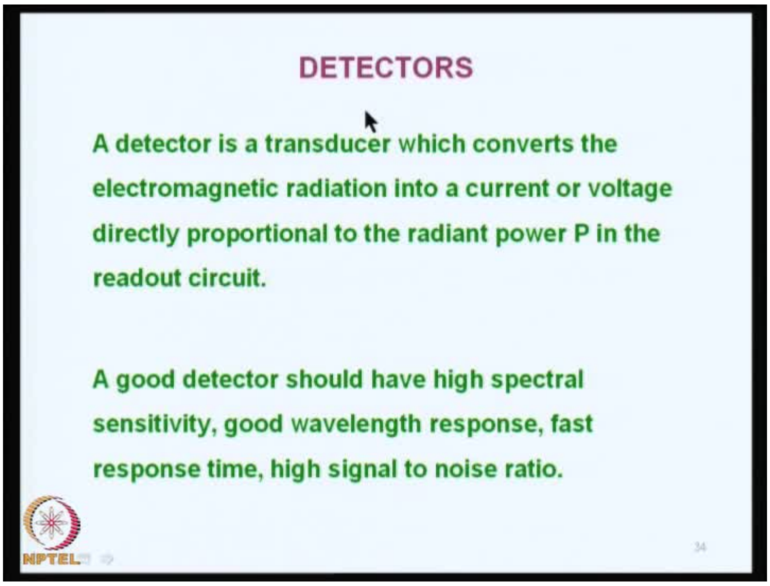

Now, this tell us that bring us to the next part of the instrumentation that is detectors. Now, what is a detector? A detector is basically a transducer which converts the electromagnetic radiation into a current or voltage and it should be directly proportional to the radiant power P in the read out circuit.

So, basically what we are looking for is information comes in through the electromagnetic radiation, in the absorption cells the sample absorbs the radiation, what comes out is the radiation of lower intensity at a particular frequency. And this radiation falls onto the detector. And the job of the detector is to produce a current proportional to the radiant power that is falling on them. If you put a blank, more radiation will fall on the detector, more light has to fall, more current or voltage is produces. As the sample content increases, the current or voltage would be less because part of the radiation is absorbed and the current will be less or voltage will be less. And this is the output is used for a read out. And a good detector, what are the qualities? A good detector should have high spectral sensitivity, good wavelength response, fast response time and high signal to noise ratio.

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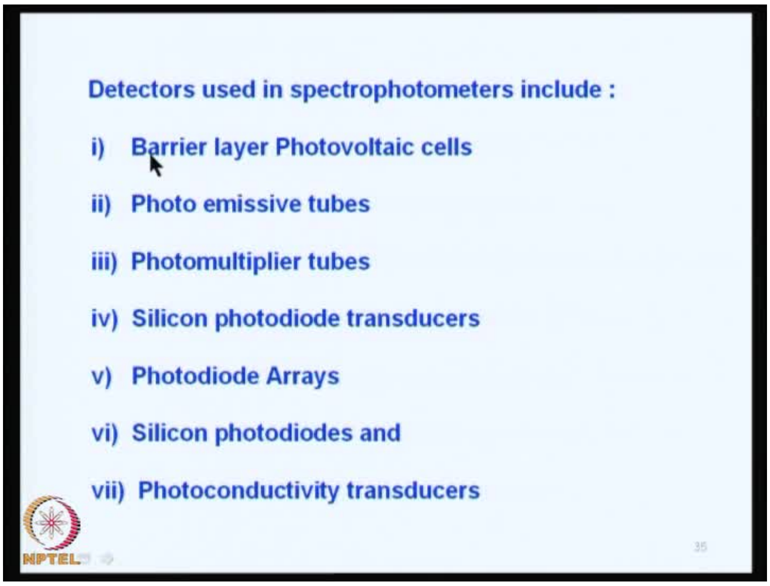

So, there are a number of detectors used in to spectrophotometers. This includes barrier layer photovoltaic cells, photo emissive tubes, photomultiplier tubes, silicon photodiode transducers, photodiode arrays, silicon photodiodes and photoconductivity transducers. In the spectrophotometer, only the these three, that is barrier layer, photo emissive, photomultiplier tubes and photodiode arrays, these are very useful in spectrophotometers. And we will discuss the construction and operations of these things in the next class.